Shank3 Gene Activation Mediated by Zinc Finger Transcriptional Activators (ZFA) as a Therapeutic Approach for Phelan-McDermid Syndrome

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Introduction

- Phelan-McDermid syndrome (PMS) is a rare genetic condition characterized by clinical features with varying severity, including intellectual disability, absent or delayed speech, and autism spectrum disorders (ASD)[1]. PMS is caused by a deletion or structural change in chromosome 22 or a pathogenic variant of the *SHANK3* gene.
- *SHANK3* encodes the synaptic protein SHANK3, localized at excitatory synapses. SHANK3 plays a major role in organizing scaffolding proteins, which are crucial for proper synapse formation and dendritic spine maturation.
- Mutations or loss of a *SHANK3* allele due to copy number variations can lead to *SHANK3* haploinsufficiency, causing synaptic and circuitry deficiencies associated with PMS[1,2].
- We designed Zinc Finger Activators (ZF Activators, or ZFAs) targeting the mouse *Shank3* gene and assessed *Shank3* mRNA and SHANK3 protein levels in cultured mouse cortical neurons and in vivo. ZF Activators are obtained by tethering Zinc Finger Proteins (ZFPs) to a transactivation domain (Fig. 1).

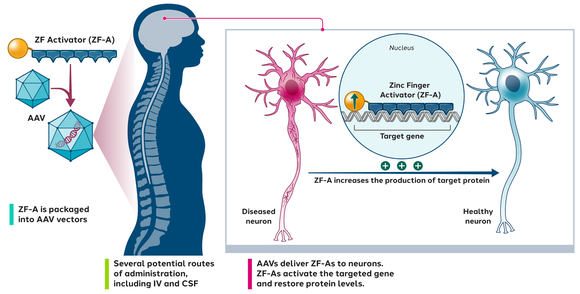


Figure 1: Schematic representation of the ZFA platform, using AAV as the delivery vector, upregulating expression of *Shank3* in neurons to rescue haploinsufficiency phenotypes.

Study Design

Candidate Shank3 ZFAs manufactured for AAV viral delivery were tested in vitro and in vivo. Downstream readouts included RNA and protein on-target activity, as well as specificity analyses by examining off-target profiles using a microarray platform (Fig. 2).

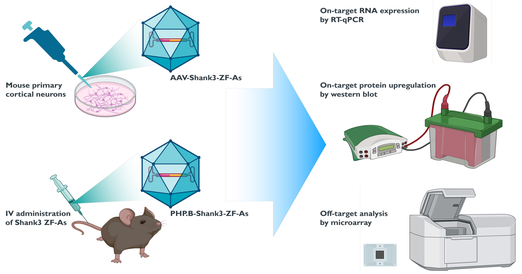


Figure 2: Schematic representation of the workflow examining the efficacy of ZFAs in vitro and in vivo. Mouse cortical neurons were transduced with AAV harboring Shank3 ZFAs.

- For in vitro studies, cultured neurons were harvested 7 days post-transduction to assess RNA and protein on-target activity and off-target analysis by microarray.
- For in vivo studies, brains were collected from adult mice 4 weeks after treatment with PHP.B encoding Shank3 ZFAs via intravenous (IV) tail vein injection.

Figures created using BioRender.com.

Results: SHANK3 ZFAs are highly specific and achieve upregulation in cortical neurons in vitro

- We designed and assembled SHANK3 ZFAs targeting DNA regions within the *Shank3* locus. Assembled ZFAs were screened for on-target activity in Neuro2A cells, leading to the identification of several ZFAs able to upregulate *Shank3* (data not shown).
- A subset of ZFAs with a range of on-target activity were manufactured in AAV and tested in cultured cortical neurons dissociated from wildtype and Shank3$^{\Delta C21}$ heterozygous mice for on-target (WT and HET) and specificity (WT) (Fig. 3).
- Shank3$^{\Delta C21}$ mice lack exon 21, causing the production of a truncated SHANK3 protein due to a missing C-terminal region. Mice heterozygous for this allele (Shank3$^{+/\Delta C21}$ or HET) exhibit behavioral phenotypes indicative of ASD. Thus, these mice are used to study ASD, particularly Phelan-McDermid Syndrome[3].

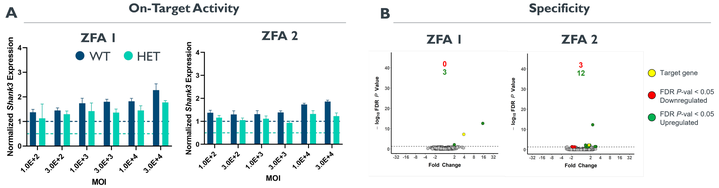


Figure 3: ZFAs demonstrate varied on-target activation and high specificity in cultured mouse cortical neurons.

(A) Bulk RT-qPCR analysis of cortical neurons after transduction of AAV-Shank3 ZFA show dose-dependent upregulation of *Shank3* expression in both WT and HET mouse cortical neurons. Error bars indicate standard deviation (SD).

(B) Microarray analysis was performed to assess the specificity of each ZFA construct in WT mouse cortical neurons 7 days post-transduction. ZFA 1 and ZFA 2 demonstrate minimal differentially expressed genes (DEGs) indicating high target gene specificity.

Results: Alternative ZF-transactivation domains (TADs) activate *Shank3* expression in mouse cortical neurons in vitro

A subset of ZFAs able to upregulate *Shank3* were used to design and assemble SHANK3 ZFAs with alternative TADs. These were manufactured in AAV and tested for on-target and specificity in WT mouse cortical neurons in vitro.

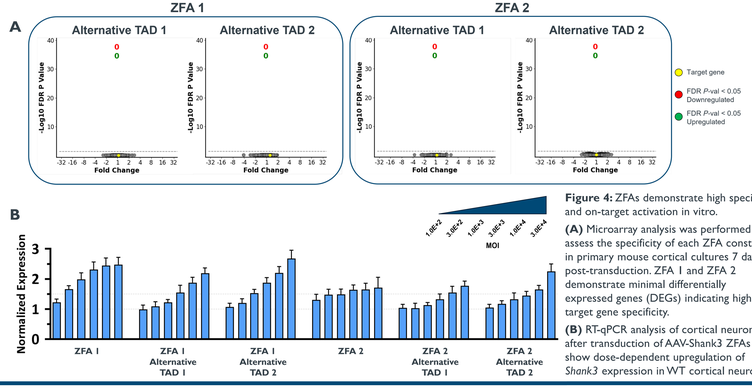


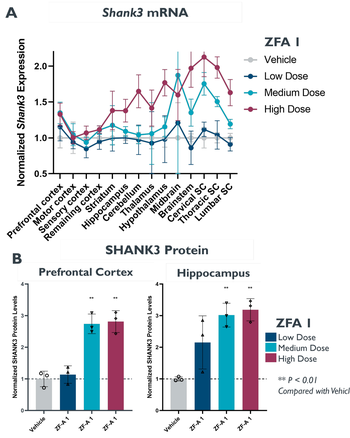
Figure 4: ZFAs demonstrate high specificity and on-target activation in vitro.

(A) Microarray analysis was performed to assess the specificity of each ZFA construct in primary mouse cortical cultures 7 days post-transduction. ZFA 1 and ZFA 2 demonstrate minimal differentially expressed genes (DEGs) indicating high target gene specificity.

(B) RT-qPCR analysis of cortical neurons after transduction of AAV-Shank3 ZFAs show dose-dependent upregulation of *Shank3* expression in WT cortical neurons.

Results: ZFAs demonstrate up to 3-fold SHANK3 upregulation in vivo

- We examined whether SHANK3 ZFAs can upregulate target mRNA and protein expression in vivo.
- In this study, PHP.B vectors encoding Shank3 ZFA constructs were delivered intravenously in a 6-week-old C57BL/6J wildtype mouse. Four weeks later, *Shank3* mRNA and SHANK3 protein levels were assessed from bulk and sectioned tissues.
- Upregulation of *Shank3* mRNA was detected in various regions of the brain. We notably observed a significant increase in SHANK3 protein levels in ASD-relevant forebrain regions, the prefrontal cortex and hippocampus, confirming ZFA target engagement.



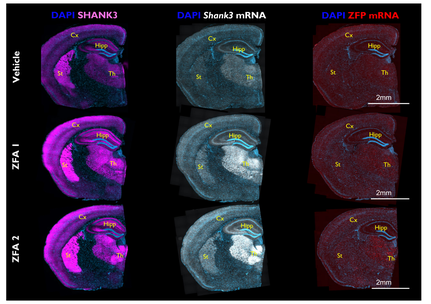


Figure 5: ZFAs upregulate target mRNA and protein expression in C57BL/6J wildtype mice.

(A) Bulk RT-qPCR analysis demonstrates in vivo target engagement and upregulation at various doses following Shank3 ZFA1 administration. Error bars indicate standard deviation (SD).

(B) Western blot analysis of protein lysates from the prefrontal cortex and hippocampus demonstrated a dose-dependent and up to 3-fold increase in SHANK3 protein following Shank3 ZFA1 administration. Error bars indicate standard deviation (SD).

(C) IHC/ISH analysis demonstrated ZFP-mediated increase in *Shank3* mRNA and protein signal in various regions, including the cortex, hippocampus, striatum, and thalamus.

Conclusion

- SHANK3 ZFAs can successfully mediate target gene upregulation in a dose-dependent manner in cultured cortical neurons from both wildtype mouse and a PMS mouse model.
- SHANK3 ZFAs successfully achieved target engagement, particularly in brain regions implicated in Phelan-McDermid Syndrome or Autism Spectrum Disorders, suggesting therapeutic potential for SHANK3 ZFAs.
- These data support further development and investigation of ZFAs as a therapeutic option for neurodevelopmental disorders caused by genetic haploinsufficiency.

References

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